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Studies on morphogenesis and development of the ascocarp of Sphaerotheca castagnei

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Submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy in the Faculty of Pure Science, Columbia University

NEW YORK
1927

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Reprinted, without change of paging, from BULLETIN OF THE TORREY BOTANICAL CLUB 54: 383–417. 5 figs., pl. 28, 29. 1927.

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Illo Hein

(with five text figures and plates 28, 29)

The structure of the ascocarp and the stages in its development have been fairly well understood since the time of De Bary, and the main points in his conclusions have been many times confirmed. The perithecial tissues are formed by aggregation of hyphae. De Bary characterized such structures as pseudoparenchymatous, and the term has been generally accepted. Little attempt has been made, however, at a morphogenetic analysis of the various growth factors, the hyphal contacts and pressures and the environmental stimuli by which the development of such structures is initiated and controlled.

LITERATURE

The literature concerning the morphology and development of the ascocarp of the mildews has been many times reviewed and analysed. I shall endeavor to summarize the data which relate to my own special problems under the following heads: (1) Pseudoparenchyma, morphogenesis, and tropistic responses; (2) Physiological species in the mildews; (3) Spore discharge; (4) Epiplasmic granules in the ascus.

Pseudoparenchyma, morphogenesis, and tropistic responses. The development of parenchyma-like tissue from filamentous hyphae was first studied by De Bary (1863, 1870) in *Erysiphe* and in *Eurotium* and by Woronin (1869–70) in *Sordaria*. De Bary described in some detail the formation of such pseudoparenchymatous tissue in the development of the perithecia of *Podosphaera tridactyla*, *Sphaerotheca castagnei* and in *Eurotium glaucum*. As is well known for ascomycetes generally the ascogonium becomes covered with an inner and an outer layer of parenchyma-like tissue which is formed by the growth, branching, and interweaving of hyphae. The hyphae divide only by transverse septa

and ultimately fill in all inter-hyphal spaces, forming an unbroken tissue. Bauke (1876) distinguished three methods of pycnidial development to which Zopf (1881) gave the names, "Hyphenfrucht," "Gewebefrucht," and "Knäulfrucht." It has been claimed by De Bary (1884), von Tavel (1886), Schnegg (1915), and Kempton (1919), and others that the walls of certain pycnidia develop by division in three planes (meristogenetic) while others are formed symphyogenously by the interweaving of hyphae and hence consist of pseudoparenchyma. The "Hyphenfrucht" and "Gewebefrucht" of Bauke and Zopf are variations of De Bary's meristogenic types. Kempton (1919) concludes that the meristogenous method is the more common and is of two types, simple and compound, while the syphyogenous method is less often found.

Hoffman (1856), Hartig (1874), Göbel (1902), Fayod (1889), and many other later students have reported detailed studies of the morphology, growth, and development of the fleshy fungi but have taken little account of the morphogenetic factors involved.

From a study of *Agaricus campestris*, W. Magnus (1906) concludes that certain specific, organized parts of the pseudoparenchyma create growth inhibitions against the organism as a whole, so that certain tissues of the sporocarp give rise in regeneration to similar tissues only; for example, the hymenium can give rise only to hymenial tissue. The regeneration of a normally lamellate hymenium may result, however, in a decidedly toothed, tubular or netlike hymenium. This condition is not to be regarded as atavistic in the Weismann sense, even though such forms are according to present phylogenetic views lower, but is due to the mechanical conditions under which the regenerative growth takes place. Transformation of vegetative to reproductive tissue results in a loss of the capacity for regenerating the whole sporocarp. So long as the tissue is still vegetative it possesses in a high degree the capacity to regenerate itself, but at a certain stage it becomes reproductive and loses its capacity for regeneration. W. Magnus also holds that the exhaustion of foodstuffs in the substratum as well as the transition to aerial growth must be of importance in determining the initiation of sporocarp formation. That the transition to an aerial environment is insufficient, as W. Magnus notes, to cause sporocarp develop-

ment is shown by the fact that in dark, poorly ventilated cellars the mycelium may grow upon the surface of the substratum for days without producing sporocarps. Mere transition from growth below the substratum to aerial growth does not suffice to initiate the development of fruit. Similar conditions were observed under bell jars placed over cultures. The hyphae grew profusely under the bell jars without producing sporocarps, while immediately outside the jars numerous sporocarps developed. Light seems to be unnecessary for the development of *Agaricus campestris*, growth and differentiation going on equally well in darkness.

Atkinson (1906) recognizes certain factors in the development of the pileus of *Agaricus campestris* as follows: "The growth of the pileus which at first is strongly hyponastic becomes less so as the pileus expands. The upper surface gradually ceases to grow and the extension of the underlying part often tears the cuticle of the pileus into fibrous scales. The growth of the pileus gradually becomes epinastic, as the lower area and the hymenophore with the gills now grow more rapidly than the middle and upper portions. This causes the pileus to become plane, or in old specimens the margin itself becomes upturned." De Bary had already described the later growth stages of the pileus as epinastic.

The geotropic responses of the sporocarps, as they occur in nature, have long been known. Brefeld (1883), Buller (1909), and W. Magnus (1906) have reported important experimental results on the geotropic and heliotropic behavior of many species. Buller (1909) reports that the stipe is indifferent to geotropic stimuli before the pileus is developed, after which it becomes positively heliotropic and rectipetal in darkness. Buller states that the position of the gills is always very delicately adjusted by geotropic stimuli. The positively geotropic response is of great importance for successful spore liberation, since these are discharged by dropping downwards and easily lodge on the gill lamellae if the latter are not exactly vertical.

In petri dish cultures of *Lasiobolus pulcherrimus* Delitsch (1926) finds that the main hyphae of the mycelium grow radially from the spore, and the secondary directions of growth are thought to be determined largely by the tendency of the hyphae to enter areas unoccupied by other hyphae.

Physiological species in the mildews. Apparently P. Magnus (1898) first published the results of infection experiments with parasitic Erysiphaceae. P. Magnus reported the transfer of *Sphaerotheca castagnei* (Lev.) from the hop to *Taraxacum officinale* but his results were not confirmed by Neger (1902). Salmon (1904, 1907) found that *Sphaerotheca humuli* from *Potentilla reptans* would not infect *Agrimonia Eupatoria, Alchemilla arvensis, A. vulgaris, Fragaria, Poterium officinale,* or *Spirea ulmaria,* all of which are hosts for *Sphaerotheca. Sphaerotheca humuli* var. *fuliginea* from *Plantago lanceolata* would not infect *P. major* or *Taraxacum officinale;* and *Sphaerotheca humuli* var. *fuliginea* from *Taraxacum* would not infect *Fragaria, Plantago media,* or *P. lanceolata,* all of which are hosts of morphologically similar forms of *Sphaerotheca humuli.*

Steiner's (1908) results from experiments with *Sphaerotheca humuli* within one host genus, *Alchemilla,* led him to assume the existence of "kleine biologische Arten." Reed (1908) has summarized the literature of inoculation experiments with the mildews and states that in his opinion most of the results rest upon inadequate data. Woronichine (1914) failed to obtain infection on peach leaves with *Sphaerotheca pannosa* from the rose and failed also in the reciprocal experiment, though the forms on both plants are morphologically indistinguishable.

De Bary's morphological work on *Sphaerotheca castagnei* was done on material from *Taraxacum officinale.* Harper (1895) and Dangeard (1896) worked on material from the hop, and Winge (1911) worked on a *Sphaerotheca* from *Melampyrum* in Sweden. It would appear probable from the studies noted that there are many physiologically different races of *Sphaerotheca* as well as of other mildews, and Gäumann's work on other fungi suggests the possibility that there may be structural differences paralleling the physiological specificities.

Spore discharge. In *Sphaerotheca* and the other mildews spore discharge involves, first, the rupture of the cleistocarp and, second, the escape of the spores from the ascus. The rupture of the cleistocarp was first described for the Erysiphaceae by Wolff (1872), later by Smith (1884) for *Erysiphe graminis,* and by Salmon (1903) for *Sphaerotheca.* The mechanism of discharge from the ascus has been frequently reported on for the club-shaped asci and has been fully described by Buller (1909) for a

number of forms. De Bary states that in the Erysipheae spore discharge is simultaneous. Wolff (1877) finds that in *Erysiphe* the spores are liberated through a longitudinal slit in the apex of the ascus. Smith (1884) found that in *Erysiphe graminis* spores and sometimes asci are ejected forcibly from the ascocarp. Salmon (1907) describes the process as observed in *Erysiphe graminis*. The spores after the ascocarp has undergone a resting period are ejected forcibly into the air to a distance of 2 cm. from the ascocarp. According to Salmon the perithecium opens by a horizontal slit at one side somewhere about the equatorial plane. This slit gradually extends until the upper half is set free like a lid. Salmon states that the dehiscence is probably brought about by the swelling of the inner layer of the perithecial cells. In *Sphaerotheca* a vertical slit appears in the perithecium and the ascus protrudes from it, swelling to several times the diameter of the perithecium. The walls of the ascus become tense, the ascospores collect at the apex, and are finally discharged forcibly through a slit. Occasionally an ascus slips out of the perithecium, ultimately bursting to discharge the spores. Recently Ziegenspeck (1926) has given an adequate review of the literature and has made a detailed study of spore discharge of many different forms of Ascomycetes, but no data as to spore discharge in the Erysiphaceae are given.

Epiplasmic granules in the ascus. Most of the literature has been summarized by Guilliermond (1904) and by Pénau (1912). Zopf (1887) described disc-shaped granules and granules of variable shapes, generally six to eight microns in greatest diameter, in the conidia, conidiophores, and germ tubes of species of *Erysiphe*, *Podosphaera*, and *Sphaerotheca* which he believed to be reserve carbohydrates and called "Fibrosinkörper." Maire (1905) described metachromatic granules in Ascomycetes and Basidiomycetes and believed them to be not particular kinds of granules, but granules belonging to the vast class of Altmann's granules which are very variable chemically, depending on the species of plant in which they occur.

The granules described by Guilliermond (1904) in the epiplasm and the sporeplasm of *Aleuria cerea*, *Helvella sulcata*, *Ciboria uliginosa*, *Pustularia vesiculosa*, *Otidea onotica*, *Peziza venosa*, and other *Peziza* species stain bright red with Unna's polychrome blue after fixation with picroformol. Hemalum

gives clear differentiation of the granules with alcohol as a fixative, but is very poor when used with picroformol.

Meyer (1904) describes, in *Penicillium glaucum*, *Aspergillus glaucus*, and the Basidiomycetes, algae, gymnosperms, and angiosperms, granules, similar to those which he earlier described in bacteria and called volutin. In unstained living *Penicillium* and in *Aspergillus* the granules can be recognized only with difficulty but with methylene blue (1 to 10) they stain dark blue in color, darker than any other parts of the cell. With carbol fuchsin the granules stain a deep red color. Delafield's haematoxylin (10 drops in 5 cc. water) produces a dark blue color when the granules are treated with carbolic acid, but when Flemming's fixative is used pale-blue stained granules result. Fresh material, as well as material treated with formalin plus 3 per cent acetic acid, when stained with alcoholic or aqueous solution of ruthenium red gives red-brown colored granules. Meyer states that the granules are present in all parts, from the germinating conidium to the newly produced spore of the vegetative plant of *Penicillium glaucum*, but their occurrence in the ascus is not mentioned.

OBSERVATIONS

The development of a three or more layered pseudoparenchyma of cells progressively differentiated from the surface inward and enclosing a single large cell, the ascus, as we find it in *Sphaerotheca*, or a group of such cells as in *Erysiphe*, may be regarded as typical for all the powdery mildews.

The mycelium

The general facts as to spore germination and the nature of the mycelium are well known but may be reviewed briefly as an introduction to the problems involved in the formation of the pseudoparenchymatous perithecium. The conidia on germinating give rise, generally at somewhat equal distances and in a horizontal plane, to three or four hyphae which grow out at right angles to the ellipsoid spore. At first the growth is very irregular but later tends more to be radial from the spore as a center. The hyphae are at first thick and tend to be nodular. Later they become more evenly filamentous in form (text figure 1, *1* and *2*). The patches of mildew generally are circular in out-

line, and examination with the lens shows that the general direction of growth of the hyphae is radially from the center. The mycelium is profusely branched, septa occur fairly regularly, and the cells are from 40 to even 80 microns in length. The average length of the mycelial cells is about 55 microns, which is similar to the average given by Bezssonoff (1914) for the mycelial cells of *Sphaerotheca mors uvae*, which he states are 57.6 by 5.4 microns. Where the mycelial network is especially well developed, septa are frequently rather close together, so that some cells are but 10, 15 or 20 microns long. The first septum is often formed a short distance, one to three microns, from the spore (text figure 2, *3*). The hypha stains pale blue with the triple stain and Allen-Bouin fixation, while the cytoplasm of the spore stains a darker blue.

The factors determining the distribution of the mycelium include (1) those concerned with the general radial distribution of

Fig. 1. *1* and *2*. Germinating conidia with characteristic irregular, gnarled germ-tubes and radially growing mycelium.

the hyphae about the spore; (2) those which determine their contact relations with the host surface; (3) those which determine the angles that the successive sets of branches make with each other.

As noted, the spore on germinating sends out from one (text figure 1, *1*) to three (text figures 1,*2* and 2,*3*) germ tubes, some of which may be very stout, gnarled, irregularly shaped structures from which other similar irregular hyphal branches or the characteristic mycelium may arise (text figure 1, *1* and *2*). A hyphal complex is thus formed; and about this there is a somewhat radial orientation of the profusely branched hyphae. The branches may arise either slightly below the septum or nearly midway

between two septa. The problems as to the direction of growth and method of branching of the hyphae here are essentially similar to those involved in the distribution of roots and branches in the higher plants. Chemotropic stimuli depending on the distribution of the food supply may be significant in determining the distribution of the ultimate hyphal branches. Whether moisture in hygroscopic relations or contact in thigmotropic relations determine the close adhesion of the hyphae to the host leaf is not easy to determine. The appresoria from which the haustoria arise are adhesion organs, and it seems probable that in some degree their development is regulated by tactic stimuli.

A fact that tends to support the assumption of hygromorphoses on the part of the hyphae is the very frequently profuse growth of the fungus in the grooves formed along the juncture of veins and leaf lamina.

In field plants the mildew first makes its appearance on the under surface of the leaves, where because of shade, more numerous stomata, and thinner cuticle the vapor pressure in the thin film of atmosphere immediately surrounding the leaf is probably higher than on the upper surface. In green-houses, where the absence of strong air currents tends to permit greater vapor pressure on both leaf surfaces, and where the general atmosphere tends to be more moist, the difference in vapor pressure between upper and lower surfaces may be less important. On green-house plants the mildew generally appears first on the upper leaf surfaces, perhaps because more spores lodge there and, finding favorable conditions, germinate and grow in situ. Eventually in the green-house the mildew also appears on the under surface of the leaf. The whole complex of factors influencing infection probably favor the under side of the leaf in field plants and the upper side in green-house plants.

That the mycelial hyphae may be reciprocally negatively chemotropic is to be considered. From his observations on *Lasiobolus pulcherrimus* grown on solid media in petri dishes Delitsch (1926) holds that the growth form of the mycelium is due to the dominately radial growth in the main hyphae and to mutually repellant chemotropic influence between all hyphae. Müller (1922), from observations made on the *Saprolegniaceae*, also suggests negatively chemotactic response as causing hyphae to grow apart from one another.

After the infected area is older, the later formed hyphal branches grow in all directions, filling in unoccupied areas. The typical radial distribution may be based on morphaethesia in Nöll's (1900) sense—that is, "Empfindungsvermögen für Form und Lage des eigenen Körpers." The deviation from the typical will be the result of various morphotic responses in Pfeiffer's (1925) sense.

Development of the primary envelope (cortical layers of the perithecium)

The term *perithecium*, though loosely used to designate the entire fructification, asci, enclosing envelope, etc., is properly used to designate only the enclosing hyphal layers, whereas the whole fructification is the *ascocarp*. I shall distinguish the outer protective cell layers as the cortex to distinguish them from the inner nutritive zone.

While the data are fragmentary and the methods of morphogenetic analysis for the thallophytes are as yet very poorly developed, it would seem that with the mass of structural and morphological material already at hand a beginning in the attempt to recognize the form-determining stimuli in the case of the ascocarp should be made. The work of W. Magnus (1906) and of Buller (1905) on the carpophore of the Basidiomycetes is suggestive of what can and should be done for the Ascomycetes.

The ascocarp begins its development after fertilization has been accomplished. There can be no question that the ascocarp is, however, strictly gametophytic in its origin. Brefeld's (1883) and Dangeard's (1896) contention that the sex cells are not functional has failed of confirmation so far, but in any case there is no doubt as to the relations of the gametophyte and sporophyte in the makeup of the ascocarp.

The enveloping hyphae arise just below the septum which cuts off the oogone from the basal cell and at a corresponding level from the basal cell of the antherid. These enveloping hyphae closely follow the contour and direction of growth of the ascogone rather than that of the antheridial stalk cell. The stalk cell of the latter forms one of the series of enveloping elements. This discriminitive relation in the behavior of the enveloping hyphae with reference to the sex elements becomes

more and more emphasized as the primary layer develops. Harper (1905) shows that the antheridial cell in *Phyllactinia* becomes embedded in the layers of the perithecium, where it ultimately gelatinizes and degenerates. In the *Sphaerotheca* here described, the antherid and its stalk cell become a permanent part of the primary envelope, in which they may be readily distinguished from the hyphal cells (figure 19). In late stages the antherid and the stalk cell cannot be identified with certainty. In all probability these cells have expanded laterally during the distention phase of the perithecium and have come to resemble closely the other cells. The special relations of the enveloping hyphae to the oogone suggest the existence of chemical stimuli with close adhesion phenomena due perhaps to thigmatropism.

All the vegetative cells of the ascocarp manifest a strong tendency to become closely appressed to one another and to the sex cells, as is shown in both early and late stages by the flattening of their surfaces of contact. The tendency of the tips of the hyphae to be pointed with the apex drawn inward toward the surface of contact (figures 7, 12 and 13) is conspicuous and significant of the physical effects of close adhesion. That the tapered form of the tips of the young hyphae of the first enveloping layer is due to adhesion with the ascogone is obvious, since the apices of other hyphae that are quite free of any contacts are well rounded, tending to a least surface configuration. The total absence of inter-hyphal spaces and general appearance of compactness of the young ascocarp are also to be noted.

The vegetative hyphae in their upward growth tend to form a uniform palisade clinging to the oogone until they meet at the apex. Since generally they do not all begin growth simultaneously, but are formed in irregular succession and are of varying ultimate heights, the final arrangement at the apex generally shows little regularity. As noted, the basal cell of the antherid contributes enveloping hyphae as well as the basal cell of the oogone and in my preparations generally, and perhaps always, produces two hyphae in addition to the antheridial branch. Text figure 2, *4*, shows a characteristic view of the enveloping hyphae that arise from the antheridial basal cell. The cell farthest to the left (text figure 2, *4a*) represents the antheridial stalk cell, the apical part of which, with the antherid, is shown in text figure 2, *5a*. The middle, somewhat shorter and younger

hypha (text figure 2, *4b*) is already binucleated. The shortest hypha is the youngest and last formed of the three (text figure 2, *4c*). These hyphae all have a diameter of about 4 to 4.5 microns; the ascogone at this stage has a length of about 17 microns. The hyphae next to the antheridial stalk cell, as in text figure 2, *4b*, begins its growth immediately to the side of it, and is from the beginning in close contact with both oogone and antheridial stalk. It will therefore follow the groove whose course is the resultant of the contours of the oogone and the adjoining cell wall of the antheridial stalk. The antheridial branch, being considerably less in diameter, following the contour of the ascogone, forms a curve with a slightly lesser radius than that of the

Fig. 2. *3*. Germinating conidium with only one gnarled germ-tube and two germ-tubes which resemble the mycelium. *4–6*. Three successive serial sections of a young ascocarp whose two-nucleated ascogone has not yet been completely covered by the initial envelope. *4a*, Antheridial stalk cell; *4b*, bi-nucleated enveloping hypha; *4c*, youngest of the three enveloping elements; *5a*, antherid with part of the stalk-cell; *5b* and *c*, enveloping hyphae. *7*, *8*. Two successive serial sections of a young ascocarp in which the initial envelope is complete and the hyphae which will form the second layer are pushing up between the two-nucleated ascogone and the initial envelope.

ascogone. The next branch will follow the perithecial wall. In general we may say, however, that the number of enveloping hyphae is seven or eight, and that apparently each hypha divides transversely but once, forming thus 14 or 16 cells. These cells are ultimately of approximately equal size, about 20 microns long, and 5 microns in greatest diameter (text figure 3, *17*), so that there are about sixteen somewhat similar cells in the first layer of the perithecium.

These cells constitute what we may call the initial envelope of the young ascogone. In spite of variations it seems to me that we can recognize elements and processes which are typical in the development up to this stage and that the considerable range of diversity in a series of young ascocarps is to be considered as a matter of morphotic modification.

The type process then may be conceived as consisting in the development of five enveloping hyphae arising simultaneously from the basal cell of the ascogone (text figure 2, *5* and *6*) and two from the basal cell of the antherid (text figure 2, *4*). Such a typical stage taken from living material is represented in text figures 4, *19–21*, and 5, *18*. The hyphae thus form a palisade of similar cells about the ascogone, the older previously formed antheridial stalk cell forming part of the series. These hyphae would typically all reach the antherid at the apex at the same time, and the initial envelope would consist of a palisade of similar cells about the ascogone. De Bary's (1863) figure 11, plate IX, for *Erysiphe* suggests such a type. If each of these cells now divides once in its middle and then expands laterally with the growth of the ascogone, we should have 16 truncated pyramidal cells. If there were as many as nine or ten original enveloping hyphae, the initial envelope would consist of eighteen or twenty cells.

No two perithecia are entirely alike in the arrangement and shape of their peripheral cells at maturity. The ultimate number of cells in the outer layer of the mature perithecium is between 18 and 24, so that it is clear that there is little cell division once the ascogone is covered, and probably some of the first-formed 16 cells do not divide further.

Whether the additional cells arise from further upgrowing hyphae or by division of the typical 16–18 cells is not clear. They grow in their tangential diameter with the growth of the ascogonium, the tangential expansion taking place at the expense of their radial diameters. Ultimately in the mature perithecial wall (text figure 5, *23*, *24*) they are plate-like, four-, five-, and six-sided or irregularly shaped cells. The cells are about 6 microns in radial diameter by 6 microns in tangential diameter when the three layers of the perithecium are complete; but when the ascocarp has reached maturity, they are generally 5 microns or less in thickness by 20–30 in tangential diameter. The average tangential diameters of the cells of the initial envelope are between 20 and 24 microns. The cells have increased about 12 to 15 times in volume.

The fertilized egg nucleus divides very shortly after fusion, and the two daughter nuclei separate slightly (figures 15 and 16) and come to lie in the upper part of the ascogone (figure 17).

At this stage the ascogone is not yet completely covered and the cells of the initial envelope have not yet begun to flatten. This binucleated stage of the ascogone is apparently of rather long duration, since the greatest growth and development of the perithecial cells takes place during this period. The ascogone does not increase noticeably in diameter, but elongates considererably during the binucleated stage, the elongation apparently taking place in the lower end while the nuclei remain in the upper end (figure 17). The ascogone, which at the beginning of the formation of the initial envelope measured between 8 and 10 microns in thickness, remains so up to the four-nucleated stage, but it elongates from 18 to 30 microns. The ascogone elongates with the growth of the enveloping hyphae. However, as it becomes enclosed at the upper end with its further elongation, it becomes curved. The initiation of this curvature

Fig. 3. *9–13*. Five successive serial sections of a young ascocarp showing the typical, irregular habit of growth of the enveloping hyphae after the initial layer is complete. *17*. External view of the initial envelope; each hypha has divided once.

may take place very early, as noted above. Its further development is, however, very plainly a matter of the interplay of two factors, first, its own growth in length and, second, the resistance offered by the cells of the primary envelope. It is obvious that less resistance is offered to lateral expansion with the assumption of a globular form than would be offered to further elongation into a cylindrical form. The position of the apex of the ascogone is for a time at least fixed by its adhesion to the antheridial cell. The stalk cell of the antherid, after its function is completed, apparently does not elongate further. The result is the curvature of the elongating ascogone in such a fashion that the centre of its convex surface comes to point vertically upward and thus fixes the location of the spatial center of the future ascus.

Figure 20 represents an ascogone similar to the one represented in figure 22, but this figure shows it rotated 90 degrees on

its vertical axis. The base of the ascogone is decidedly narrowed, and suggests that the vegetative hyphae may exert considerable pressure in this region.

This narrowing of the base of the ascogone begins to appear after the first layer of the perithecial hyphae is complete (figure 17), and from the general appearance of this region it appears as if the ascogone were wedged in on all sides. This constriction becomes more pronounced, until the base has the form of an inverted cone (figures 20, 23). However, the constriction of the base of the ascogone is not always so pronounced and often appears to be quite wanting (figures 18, 21, and 22).

When the primary envelope has completely enclosed the ascogone, a second layer of hyphae begins to develop between it and the ascogone. The hyphae that form the inner layer arise from the basal cell, alternating with those of the ascogone (figures 16 and 18), and appear to wedge their way between ascogone and primary layer, becoming, as a result, somewhat wedge-shaped at their ends. In pushing upward these hyphae force the outer layer outward and away from the ascogone (figures 13, 16 and 18), so as ultimately to form a second complete layer about the ascogone.

The antherid and its stalk cell, which in the beginning were part of the initial envelope, have become a permanent part of this layer, and are separated with it from the ascogone by the intercalated secondary layer. In figure 19 the elongated antheridial stalk cell may be seen as part of the outer wall of the perithecium, the empty antherid in this case being lifted entirely above the ascocarp. This is a common appearance. The old antherid very frequently appears as though the enveloping hyphae had forced themselves under it (figures 13, 18, and 19).

With this stage the rudiments of the cortical layer are in the main complete. The ascocarp at this stage, when the two first layers of the perithecium are complete, has an outside diameter of about 35 to 40 microns (figures 18, 20, and 21). The perithecium seems to expand more rapidly in its diameter than the ascogone grows in size, so that there is a tendency to leave vacant space between the two.

Development of the sex cells, the ascogone, and the ascus

After the mycelium has reached a certain stage of maturity, the sex cells develop. They tend to grow upward, away from

the leaf surface, at angles varying around 90 degrees. They rarely stand at exactly 90 degrees to the host surface; commonly they are about 60 degrees and occasionally at 45 degrees to the substratum. I find no evidence that these cells in their growth react in any way to light or gravitational stimuli. The mildew, as stated above, is either epiphyllous or hypophyllous, and the leaves are inclined at varying angles to the sunlight. The sex cells of *Sphaerotheca* show equally varying positions, whether on one side or the other. Negative response to moisture, hygromorphosis, may be concerned in determining their general positions.

The distribution of the sex cells on the mycelium illustrates perfectly the conception that sexual reproduction is the expression of what we crudely call maturity in the life cycle of the mycelium. The sex cells are formed from hyphae about six microns in diameter, when the mycelial patches are a millimeter or more in diameter, and they appear successively as the mycelium spreads and the hyphae attain a certain maturity. At the time the sex cells appear, the hyphal cytoplasm is homogenous and only very slightly vacuolate. The assumed physiological changes of sexual maturity come to expression as a difference in staining reaction, both between antherid and oogone and between them and the vegetative hyphae.

The antheridial branch in my preparations is always in advance of the oogone in development (figures 1, 2, 3, 4, 5, and 6), or rather is always longer, for the total volume of the two cells seems to be about the same. In the stage represented by figure 5 the average dimensions of the oogones are about 7 by 15 microns, of antherids 5 by 25. The two branches adhere closely, and often it appears that the more rapidly growing antheridial branch tends to bend the oogone to one side. The preparation represented in figure 3 clearly illustrates this point. Since the oogone does not keep pace with the more rapidly elongating antheridial filament, the two bend toward the oogonial side. This may explain why the oogone and the ascogone are also so commonly inclined at an angle less than 90 degrees to the leaf surface. The study of living material shows that even the very young stages are generally at such an angle.

I am inclined to believe that the curve in the oogone, the bending over upon itself in the later stages of its development

before the formation of the ascus, is initiated by this curvature of the oogone. I shall discuss this later on.

The antheridial branch is about twice the diameter of the mycelial hypha from which it arises, and appears as an elongated, stout, somewhat pointed cell, closely appressed to the oogone and growing in the same direction with it (figure 1). The single large nucleus that is at the base of the branch divides, and a wall separates the sister nuclei, which are now about one-half the size of the original nucleus, and one remains in the newly formed basal cell (figure 3), while the other migrates to the upper part of the branch where another division takes place. The resulting nuclei have about one-half the diameter of the original nucleus of the antheridial cell (figures 5, 6, 7), though the upper one is considerably smaller and migrates to the very tip of the cell where it is cut off by a wall (figures 5, 6, 7). The resulting uni-nucleated apical cell becomes the antherid. Thus three uni-nucleated cells are formed, a small, oblong, somewhat cap-like cell, the antherid (figure 7), a longer portion whose protoplasmic content is homogenous and tends to stain more densely than that of the other cells (figure 7). The antheridial stalk cell is rather stout in the middle and tends to taper at each end with the nucleus in the middle (figures 7, and 10). The basal cell is decidedly vacuolate (figure 6). The whole filament closely follows the contour of the oogone and its tip, the antherid, lies more or less on the apex of the latter (figure 5, 7, 10). Occasionally the antherid is placed a little to one side of the apex, as shown in figures 8 and 11.

The oogonial branch is generally about three times the diameter of the hypha from which it arises, and appears as a short, stout, somewhat ovoid uni-nucleated branch. Occasionally an oogonium appears slightly narrowed towards its apex, as in figure 4; but I am not certain that this is not due to the treatment by fixatives. The oogonial branch contains near its base a single large nucleus, which is about 5 microns in diameter, slightly larger than that of the nucleus of the branch destined to produce the antherid (figure 1). The oogonial nucleus divides, and a wall separates the daughter nuclei (figures 2, 3). The cross wall is marked by a slight constriction and is placed about one-third of the distance from the base of the oogonial branch to its apex (figures 2, 3, 4). The upper two-thirds of the branch becomes the oogonium, which at this stage is markedly turgid,

ellipsoid, and thin-walled. The cytoplasm of the oogone at this stage may be homogeneous or very finely granular, and shows no vacuoles; but the basal cell begins to show very small vacuoles, even at this early stage, and its cytoplasm is less homogeneous (figure 6). The nucleus of the oogone is considerably larger than that of any other cells of the group, measuring about 3.5 microns. The oogonium now bulges slightly at the apex, forming a papilla. The bulging region shows a strong affinity for the red stain of the Flemming triple stain. With Unna blue it stains very deeply, appearing very dark blue even in otherwise weakly stained preparations. It seems probable that there is a gelatinization of the walls between antherid and oogone in this region. Preparations of a stage a little later show a comparatively thick cell wall in this region which stains very dark red and again suggests that the wall is gelatinous at this point.

Fertilization is accomplished by the breaking down of the adjoining walls of the antherid and the oogone, followed by the entrance of the male nucleus into the oogonium and ultimately the fusion of oogonial and antheridial nuclei. The pore is most clearly visible when the antherid and oogone are viewed from the side (figures 8, 10). The cytoplasm of the two cells is seen to be continuous, though there is no evidence that the entire contents of the antherid is emptied into the oogonium. Only the antheridial nucleus migrates into the oogonium, where it moves immediately to the female nucleus.

In figure 5 the sexual cells are complete, and fusion is about to take place. The region of fusion shows the characteristic staining reaction. In figure 8 the fusion pore is distinctly seen in section, the cytoplasm of the one cell is in contact with that of the other, and the small male nucleus, which is just within the oogonium, has apparently just entered it. In figure 9 the male and female nuclei are lying side by side, and are about to fuse. Which of these two figures represents the older stage, I am unable to say. The cells shown in figure 14 are larger, and one of the first of the enveloping hyphae is beginning to grow from the basal cell. It may therefore be considered the older. The two nuclei are now about equal in size, the antheridial nucleus having grown slightly (figure 9). Both male and female cells are in this case considerably smaller, and they may be regarded as in a slightly earlier stage than that shown in figure 14. In figure 11

the two nuclei have fused completely; the nucleus here is about 5 microns in diameter, having apparently about twice the combined volume of the two figures in figure 14. In figure 10 are represented a pair of larger sex cells, in which the sexual nuclei have already fused. The fusion nucleus here has a volume about equal to that of the volume of the two sexual nuclei represented in figure 14. Figures 12 and 9 may represent successive stages of a smaller pair of sex cells than those represented in the figures 10 and 14. Such figures merely represent variations in size of the gametic pairs. A figure like that represented in figure 12 would never in the immediately following stage look like the one represented in figure 10.

The fusion nucleus now undergoes a division resulting in two equal-sized daughter nuclei, and a rapid growth of vegetative hyphae about the two-nucleated ascogonium takes place.

Fig. 4. *14–16.* Young ascocarp showing irregular development of the perithecial wall. In *14*, there is a slight tendency of the hyphae to form a spiral pattern. *19–21.* Successive sections of a one-nucleated ascogone with more or less uniformly developing enveloping hyphae.

The two nuclei of the ascogone during the development of the perithecium divide once so that there are four at about the time when the third layer of the perithecium is complete (figure 24). I am of the opinion that these nuclei arose by a simultaneous division of the two nuclei in the ascogone. I have never found a distinct three-nucleated ascogone but have many preparations which show four nuclei in the ascogone and many preparations which show ascocarps at about the same stage of development as the four-nucleated ones but with only two nuclei in the ascus. It is therefore very probable that the four-nucleated immediately follows the two-nucleated stage, the nuclei dividing simultaneously. Cross walls then separate the terminal and basal nuclei from the middle pair in the ascogone. There thus results a column of three cells, of which the middle one is binucleated and

develops to form the ascus. The binucleated central cell, the penultimate cell, is in the region of the ascogone in which the bending takes place (figure 25), so that from the outset it bulges out much more on its convex side. It expands very rapidly and soon becomes short and pear shaped (figures 26, 27, and 29).

The two nuclei in the penultimate cell move together (figure 25) and fuse (figure 26), forming the definitive nucleus of the ascus. Figure 26 represents a young ascus showing the fusion nucleus in which the nucleoli have not yet fused. The young ascus at this stage has a diameter of about 15 to 18 microns, the diameter of the whole ascocarp being around 40 microns. After nuclear fusion the ascus continues to expand very rapidly, apparently in every direction (figures 26, 27, 28), and the nucleus enlarges with it. In the beginning the nucleus is elongated, appearing to be drawn away from its basal position (figure 27). The ascus apparently becomes very turgid to conform, in spite of the irregularities in the perithecial cells and its original shape, to a least surface configuration. At its base the young ascus tends to remain narrowed as though pinched by surrounding cells for a considerable period (figures 26, 27, and 29), and in some cases this pinched condition is never entirely lost. The ascus grows rapidly and symmetrically against the pressure of the perithecial wall. Its basal portion is the last to round out and lose its blunt-pointed shape. Many asci do not become spheres, but remain slightly dorso-ventrally flattened, appearing oval in section (figures 33, and 35).

The cell wall of the ascus is very thin up to the time the spherical shape is attained, but after this there is a gradual thickening as maturity is approached. In the stage shown in figure 30 the cell wall is shown as very much thickened.

Further growth and ripening of the perithecial envelope

The inner zone of the perithecial wall develops between the ascogone and the second layer and apparently arises largely by growth of the cells of the second layer (figure 29). That is, we now have a beginning of radial growth inwardly from the envelope toward the ascogone. In figure 22 the two elongated cells in the lower right-hand side of the figure have the appearance of having elongated radially and are about to divide. Immediately above these two cells are a short and a long cell which apparently

are the result of division of a single elongated cell. Possibly the two cells below will divide similarly, and in this manner the innermost layer of perithecial cells may arise. The third layer, as noted, begins to develop at a time when the curved ascogone does not completely fill the perithecial wall. Its cells at first push into the spaces around the ascogone and show no tendency to tangential flattening. Later, when the ascus begins to swell rapidly, they are pushed back so as to form a layer of cells of fairly equal size and constant shape (figures 31, 33, and 35). On the right-hand side in figure 22 there are three cells of the inner layer which, because of their narrowness and obvious connection with the basal cell, appear to have arisen by upgrowth from it and not by ingrowth from the secondary layer. The innermost layer of cells arises both by transverse divisions of the ingrowing branches from the second layer and in lesser degree by further upgrowth from the basal cell. The ingrowing branches would typically tend to become cylindrical hyphae, but they take on such shapes as their position permits. Ultimately they are irregularly polygonal in tangential aspect and irregularly quadrilateral in radial section (figure 29). The ascocarp shown in figure 31 has almost reached its maximum size, and the cells of the outer layer are beginning to approximate their ultimate plate-like form. The inner cells are still large and isodiametric, and interhyphal spaces are present. The whole perithecium in this figure has about the number of cells commonly found when the ascocarp is mature, and probably there is no further increase in the number of the perithecial cells after this stage. With this stage the growth of the perithecium is complete, and its further modifications are those of differentiation and specialization.

After the nuclear fusion the young ascus grows very rapidly, the ultimate cell and the stalk cell of the ascogone become part of the inner layer of cells of the perithecium. These cells of the ascogone remain distinct for a time, as is shown by their different staining reaction (figures 29, 31, 34 and 35); but when the ascus expands their identity is lost and they can no longer be distinguished easily from the other cells of the inner layer.

The cells of both the outer cortical layers of the perithecium now begin to show changes in their appearance. The cell walls become slightly thickened and the contents vacuolated. I am of the opinion that after this stage they no longer divide, the

ultimate number of cells between 18 and 22 being reached at this time. As the cells grow tangentially they leave no intercellular spaces between them. The hyphal cells adhere very closely and are in intimate contact throughout. They form a strong limiting layer against which the inner cells are flattened by the inflating ascus. In figure 35 the ascus has expanded considerably beyond the size shown in figures 27 and 29, and the cells of the inner layer are becoming flattened radially. Ultimately the ascus obviously develops a high turgor pressure which flattens the cells of the softer inner layer so that they become quite thin (figures 33 and 35).

These inner radially growing cells of the perithecium retain their protoplasmic contents even to a very late stage, when the

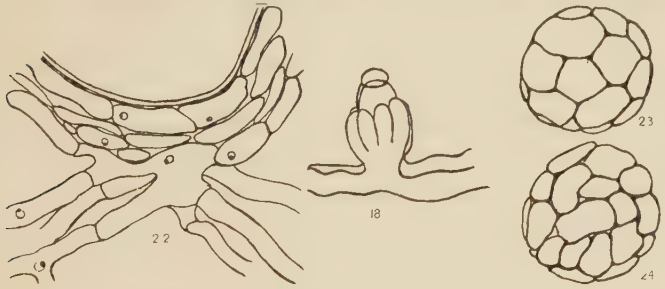

Fig. 5. *22.* Base of a mature ascocarp raised from the leaf surface by the appendages. *18.* Asocogone with palisade of enveloping hyphae growing uniformly upward. *23, 24.* Apical views of mature ascocarps showing the typical patterns of the perithecial cells.

spores are completely developed. Even in early stages they have a different staining reaction from that of the wall cells and doubtless they have a different function. They are large and rounded in the beginning and decrease in size with the growth of the ascus. The natural suggestion is that they are nurse cells for the ascus, as was noted by Harper (1895), and always remain thin walled.

The basal cell of the ascogone in these later stages is still clearly differentiated from the surrounding cells, and there is apparently a definite functional connection between the ascus and the stalk cell of the whole ascocarp (figure 31). It is, however, possible that after the ascocarp has reached its maximum diameter there is no further absorption from the substratum,

the ascus growing at the expense of the perithecial cells—probably the inner nurse layer.

During the earlier stages, when they are still large and isodiametric, the cells immediately surrounding the ascus are regularly uni-nucleated; but later, when the ascus has taken on its illipsoid shape, these cells become bi-nucleated to tetra-nucleated. The cells of the two wall layers also contain one or two, rarely three nuclei in fully developed ascocarps.

The appendages begin to appear when the three layers of the perithecium are complete. They arise from the outer layer of cells in the basal region of the perithecium. They are eight to twelve in number and are long (300 to 400 microns), nucleated, tortuous, and hyphae-like. Occasionally an appendage is branched. They interweave with the vegetative mycelium, and it is often difficult to distinguish the appendages from this mycelium. When the ascocarp is mature, it is often seen lifted up above the leaf surface to a height equal to its own diameter. Apparently it is raised above the leaf surface by the appendages which are directed downwards at an angle (text figure 5, *22*). There is no evidence of any special mechanism in the appendages which serves to raise the ascocarp from the leaf surface, such as is found in *Phyllactinia*. Apparently it is due to the downward growth of the numerous appendages.

Epiplasmic inclusions

I first observed highly refractive, conspicuous granules in the ascus of *Sphaerotheca castagnei* in material fixed with Allen's modification of Bouin's fixative. These granules stain bright ultramarine blue with the triple stain. In very late stages they stain red. Merkel's fluid, Herman's fluid, picroformol, plain Bouin and Allen's Bouin give preparations in which the granules appear bright blue when the triple stain is used. With Flemming's fixing solution such granules do not generally appear, though occasionally hyaline, refractive granules resembling them in shape, number, and location may be found. It is probably because of the very widespread use of Flemming's fixative, in which the granules are scarcely if at all differentiated, that they have hitherto been overlooked in the mildews.

Unna's polychrome blue, when used with Allen's Bouin, Merkel's or plain Bouin fixative, gave varying results. The

granules usually stained a deep violet, purple or reddish purple color, though sometimes they showed pale blue or greenish blue and occasionally would take no stain (figure 34) at all, even though other parts of the ascocarp showed well stained granules. While the granules frequently exhibited very distinct metachromasy with Unna blue, I have never obtained with this dye the bright red granules described by Guilliermond (1904) for the granules in the asci of *Aleuria cerea*, *Helvella sulcata*, etc.

The perithecial cell granules are very small, numerous, and purplish in color with the Unna dye, and appear long before any granules become visible in the ascus. These are quite different in appearance from those which later develop in the ascus, remaining small, very dense and staining dark blue with Unna blue even in very late stages, while those in the ascus become very large, less numerous, and not so dark in color.

No such granules appear in any of the cells at very early stages of the developing ascocarp. Only after three layers of perithecial cells are formed do they begin to appear, the first ones becoming visible in the innermost cells (figure 28). The perithecial cell granules are fairly numerous from the beginning and become several times larger and more numerous in later stages (figure 33). The epiplasmic granules do not appear until after nuclear fusion. Here again they are numerous from the beginning, becoming more numerous and larger as the ascus increases in size. The granules appear to be formed only after a cell has reached a certain stage of development. In cells that have just divided no granules are present, as for example in certain cells surrounding the penultimate cell represented in figure 28. Here certain cells of the third layer have no granules, while others have extremely minute, barely visible granules. The perithecial cell granules in the second and first layers are larger and more numerous, while the penultimate cell has none. It is difficult to ascertain from my preparations how these granules arise in the cells.

The epiplasmic granules, even in the early stages, are found each lying in a clear area in which they are eccentrically placed and increase many times in diameter as development proceeds, from particles barely visible with highest magnification to those as great as seven microns in diameter. When the ascus has reached its maximum diameter, the granules are from three to six microns in diameter and appear to fuse (figure 33). The

fusion appears to be merely a physical result of their coming in contact through an increase in their diameter and perhaps through cytoplasmic movement. The largest granules appear in the ascus during the uni-nucleated stage, when certain of them may attain a diameter of ten microns. After the first division of the ascus nucleus during the two-nucleated stage, there are present numerous small epiplasmic granules varying in dimensions but below 2.5 microns or very rarely 3 microns in diameter (figure 30). Such granules vary in their staining reactions; some become red, some blue with the triple stain, though all appear to stain blue when the treatment with safranin is very brief. If these granules are similar to those in the immediately preceding uni-nucleated stages, it is difficult to understand how the abrupt change in their size takes place. Large granules occur in all the late uni-nucleated stages but the immediately following bi-nucleated stages have the small granules. Throughout the four- and eight-nucleated stages as well as in certain eight-spored stages the small epiplasmic granules persist.

In stages in which the outer perithecial cell walls are brown and the protoplasts extremely vacuolated, large irregular pale blue masses, from eight to fifteen microns in diameter with from one to four dense, dark blue, spherical centers and resembling several fused, large granules of the kind found in uninucleated asci, are present in the epiplasm. They appear to be aggregates of large granules and may arise as Bunge (1895) described by the fusion of other granules. In addition to these, many small granules like those described are also present in the epiplasm as well as in the spores (figure 35).

Similar granules are common in young hyphae. In conidiophores and conidia, as well as in the germ tubes of the latter, granules of similar appearance are also frequently present.

In still later stages large, often irregular granules, six microns in diameter, both red and blue in the triple stain, occur in the epiplasm. These are probably degeneration products and unlike any of the granules described above.

In the fusion nucleus of the ascus granules similar to those in the epiplasm occur (figure 27). I have not found them in any other nuclei of the ascocarp, the large nucleus in the ascus being the only one in which they are definitely recognizable.

In the ascus during the uni-nucleated stage, the epiplasmic

granules are indiscriminately scattered in the epiplasm. There is not the slightest tendency for them to be localized at any time, either about the nucleus or in any other region of the ascus. They present no evidence of connection with or origin from the nucleus or through its agency. There are commonly, as noted, both large and small granules within the nucleus, but these are not generally in connection with the chromatin. Guilliermond's (1903) suggestion that the granules may be formed as a result of secretions from the nucleus or that they are secretion products from the chromatin finds no support from my study of *Sphaerotheca*.

Judging from their spherical form, the granules are liquid or semi-liquid substances chemically different from the surrounding epiplasm. They arise perhaps as very minute droplets secreted by the cytoplasm.

DISCUSSION

In the light of the specificity in host relations found in the mildews of P. Magnus (1898), Salmon (1904), Steiner (1908), Reed (1908), Woronichine (1914), and others and of the morphological variations which have been reported as occurring in single species, as recognized in Salmon's monograph, I am of the opinion that further study may lead to the recognition of many of the current biological races of mildews as varieties or even species. Woronichine has already reported variations in the dimensions of ascocarps, asci, spores and certain sterile hyphae of *Sphaerotheca pannosa* on the rose and of *Sphaerotheca pannosa* on the peach and on the basis of both the physiological and morphological differences proposes to recognize them as distinct varieties.

The fact that on a particular host there may be two or more asci in a form which usually has but one ascus and that the perithecium which usually consists of but three layers occasionally has four or even five layers as well as other structural variations, shows the need of further study of the species question in these fungi.

Harper (1895) describes the ascogone in *Sphaerotheca* as consisting regularly of a column of five or six cells, while Dangeard (1896) figures only three. In my own preparations I find regularly three cells in the ascogone and in only one instance a four-

celled stage (figure 38) among several hundred three-celled ascogones. That such basic structures can so vary seems hardly probable, and the need of study of further forms is obvious.

Gäumann (1926) has suggested that the lack of agreement in the conclusions drawn from observations on the morphology and development of the mildews may be due to the fact that the studies were made on material from different host species.

The great similarity between the parenchyma of higher plants and the pseudoparenchyma of fungi is striking in view of the difference in the method of their formation. Parenchyma is formed by the growth and division of the cell in length, breadth and thickness. Pseudoparenchyma is formed by the growth, branching, interweaving, and adhesion of hyphal filaments.

The special relations between the cells in a parenchyma are determined largely by their method of origin, though the evidence seems to favor the view that the contact relations of the protoplasts may be dependent on the secondary development of plasmodesmen. The interrelations of the cells in the case of pseudoparenchyma are for the most part secondary and are achieved by thigmotactic responses and adhesion. Pits and presumably plasmodesmen are present and serve possibly as conductive channels through which morphotic stimuli are transmitted from cell to cell.

The problems involved in the distribution of the mycelium on the leaf of the host are doubtless analogous to those presented by the distribution of roots in the higher plants. It is hard to conceive that negative chemotactic responses involving repulsions are responsible for the generally radial direction of growth of the hyphae about a germinating spore. I have noted that the initial growth results in a generally radial arrangement of the main hyphal branches and that the typical appearance of the mycelium on an infected area of leaf is that of a radiating system of hyphal filaments. According to Nöll (1900) the radial direction of growth of secondary plagiotropic roots after the removal of an interposed barrier is due to morphaesthesia. According to this conception the main orienting factors in hyphal growth would be matters of rectipetiosis. Further observations should be made to test this and other hypotheses as to the factors which determine the growth directions of the hyphae on the leaf.

The differences in the staining reaction between the sex

cells themselves and between them and the vegetative hyphae suggest physiological differences which may be chemical in nature. Whether these changes should be classed as rectipetioses is to be determined perhaps by Pfeiffer's (1925) limitations of the conception. Pfeiffer holds that the internal morphotic factors operate as specific substances which are produced by the tissues and are carried from cell to cell or through conducting channels to more distant tissues whose growth forms may be thus determined. The difference in staining reaction in the sex cells, as described above, may possibly be an indication of the formation of such specific substances.

It is evident that carpogenesis is initiated by fertilization, and it is natural to assume that the latter process involves the formation of specific substances which act as stimuli in initiating the outgrowth of the hyphae of the primary envelope. Such stimuli may be classed as carpogenic factors. The substance may be of the nature of hormozones in Gley's (1911) sense and in a morphogenetic sense may be called morphones.

The development of the perithecial wall does not generally begin before the fusion of the sexual nuclei, but I have found some cases in which the enveloping hyphae bud out before this stage. Such precocious development indicates that the stimuli to carpogenic development may arise before cell fusion occurs. The case represented in figure 13 is unusual. Fertilization does not yet seem to have taken place, but the enveloping hyphae are sprouting from the basal cells of both antherid and oogone. In figure 13 the hyphae are shown as already growing from the basal cell, yet fertilization has apparently not yet taken place. The antherid is gradually being pried away from the oogone by the hyphae which are pushing up between it and the initial envelope. That in this case an antherid is being pushed away from the oogone is possible. The antheridial filament is always more advanced in its development than any other hypha of the ascocarp, and typically occupies a position similar to that represented in figure 13. The case may be one of delayed or abortive fertilization in which perithecial development has none the less continued. It is to be noted that the oogonial nucleus in this case is unusually large and has not divided. Possibly figure 37 represents a still later stage of a similar case in which the large oogonial nucleus has still not divided, while perithecal development has gone on independently.

As noted, I have not observed the spiral pattern of the initial envelope of the ascogone regarded by De Bary as characteristic, and I am of the opinion that it is at least unusual and results only when there is a very rapid uniform development of the enveloping hyphae (text figure 4, *14*). The explanation of such a pattern is probably analogous to that generally accepted for the spiral twist in the enveloping cells about the egg of *Nitella;* that is, the combined transverse diameters of the enveloping cells are less than the circumference of the ascogone and their length considerably greater.

Several of Dangeard's (1896) figures are unlike any which I have observed. His figure 15a (p. 276, 1896) shows an oogone of two cells; figure 15d, a four-nucleated ascogone with little perithecial development; figure 15e is also unlike anything that usually occurs in my preparations. I have found occasionally some very unusual figures which in my opinion are abnormal, at least for the complete life history of *Sphaerotheca*. Some of these figures resemble certain of those of Dangeard, except that they are extremely large. Figures 36 and 37 are comparable to the four-nucleated stage represented in Dangeard's figure 14b, but in the former perithecial development has been inhibited. Figure 32 may represent a case in which the oogone has never been fertilized, yet has continued to grow. The filament (a) in figure 32 may be a degenerating antheridial filament which for some reason never came to maturity, possibly because it developed from an incompatible mycelium. The presence of two or three nuclei in the terminal cell of such a presumably unfertilized oogone is very peculiar and not easily explainable.

The stalk cell in these preparations (figures 32, 36 and 37) is in each case uni-nucleated. These naked oogones (they can hardly be called ascogones, since there is no assurance that asci ever develop from such structures) may on the other hand be looked upon as isolated oogones which have developed without the presence of antherids. The oogone in figure 13, where the male nucleus has apparently failed to enter the oogone, suggests the possibility that oogones and antherids may develop in contact and still fail to complete the process of fertilization.

The demonstration of the presence of a specially stained region of the wall of the oogone at the apex of the antherid suggests an analogy with the receptive papilla of *Pyronema*,

Albugo (Stevens, 1899), and certain algae, such as *Vaucheria* (Oltmanns, 1895), *Oedogonium* (Klebahn, 1892). I do not find a hyaline receptive spot in the cytoplasm beneath the stained area. It is the wall of the oogone apparently which becomes differentially stained as shown in figures 6, 10, 11, and 14.

In his studies on mildews Bezssonoff (1914) is in disagreement with earlier workers on several points. According to Bezssonoff the antheridial branch of *Sphaerotheca mors uvae* does not cut off a small uni-nucleated antheridial cell as in the species of *Sphaerotheca* described by Harper (1895, 1896, 1905), Dangeard (1896), Blackman and Fraser (1905), and Winge (1911); but the antheridial branch becomes bi-nucleated, the apical nucleus migrates into the oogone, and the remaining nucleus degenerates in situ. In *Microsphaera*, Bezssonoff claims to have a stage showing the passage of the male nucleus into the oogone, though his figure 3, plate VII, does not show this very clearly. The fusion pore differs from previously described fusion pores in the mildews in being at the extreme end of the area of contact between the antherid and oogone, their walls at this point appearing to form an almost straight line.

The initiation of perithecial development in the mildews by the formation of a circle of perithecial hyphae which grow out radially from the stalk of the ascogone, and the development between these hyphae and the ascogone of further series of hyphae, parallels in principle the corresponding stages in the development of the apothecial envelopes of *Pyronema* and other Discomycetes. The subsequent transformation of these vegetative hyphae into the variously shaped plate-like cells of the perithecium, the differentiation of the outer cortical layer into dark colored, thick-walled cells and the inner layer into thin-walled cells from which the nurse layer develops, are to be regarded as expressions of the most specialized morphotic responses so far described for any ascocarp. In the formation of the layers of pseudoparenchyma of the mature perithecium, two types of development may be recognized. The initial envelope and the inner perithecial layer may be regarded as at first a simple prosoplectenchyma which is developed symphyogenously. The mature paraplectenchyma (Lindau, 1899; p. 25; Gäumann, 1926) or pseudoparenchyma results from the transformations through thigmotactic responses of the prosoplectenchyma (Lindau,

Gäumann) into the compact mature tissue of the outer wall. I have never found any evidence of meristogeneous division at any stage in the development of the cleistocarp. The mature cells are variously quadrilateral, pentagonal, hexagonal or irregular in tangential section and very much flattened as a result of pressure from the growing ascus. Thigmotactic responses with radial pressure from the ascus are the dominant factors in determining their form.

The spherical ascus of *Sphaerotheca*, as compared with the club-shaped forms of the Discomycetes and the intermediate forms in *Erysiphe* and *Phyllactinia*, illustrate how pressure may modify the tendency to a least surface configuration. When in *Sphaerotheca* two asci are developed in a single perithecium they modify one another's form at their surfaces of contact so that the adjoining walls become flattened and the asci become approximately hemispherical. In cases where three asci are produced as reported by Salmon (1900), they probably assume a form analogous to that of the carpels of an orange. The more numerous the asci become, the greater must be their mutual lateral pressures and the more elongated and club-shaped is the form (see Harper's figure 40, plate XII, 1896).

The name nurse tissue is suggested for the innermost layer of the mature cleistocarp of the mildews because of its suspected connection with nutrition of the ascus, as indicated by Harper (1896 and 1905). Harper (1896) also suggests that this layer may be capable of swelling on taking in water, causing the ascocarp to burst through high turgor of these cells, in order to liberate the ascopores. The fact that this layer is soft and moist at the time of spore discharge and that the whole ascus sometimes slips out (Salmon, 1907) or is ejected forcibly (Smith, 1884) also suggests its connection with spore discharge. Possibly this layer functions during the growth of the ascus as a nurse layer and is later instrumental in bursting the cleistocarp.

The appendages in *Sphaerotheca* closely resemble in their origin the secondary mycelium probably present in most ascocarps and described by Harper in *Pyronema* (1900). The secondary mycelium in the case of the larger ascocarps doubtless functions in the nutrition of the later growth stages. For the relatively small ascocarps of the mildews, nutrition seems adequately provided through the stalk of the ascogone. De Bary

(1863), in his general description, states that secondary mycelium in the mildews is developed at an early stage. The morphological evidence seems to favor the view that the mildews are not primitive, but highly specialized in connection with their obligate parasitism.

With the acquisition of the parasitic habit and the reduction in size of the ascocarp, the secondary mycelium became nonfunctional. Obviously the appendages are to be regarded as vestigial secondary mycelium. There is no evidence that the appendages of *Sphaerotheca* ever develop haustoria. Their ends are in all cases free and undifferentiated. In forms of the Erysiphaceae in which the appendage endings are branched or otherwise modified they apparently are acquiring new functions. The highly specialized structure of the appendages of *Phyllactinia* is adapted for lifting the cleistocarp up from the leaf surface. This is, then, a case in which vestigial organs have become secondarily specialized and have acquired a very specific new function.

I feel deeply indebted to Professor R. A. Harper for his encouragement and valuable assistance in the preparation of this paper.

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Explanation of plates

All figures were drawn with the Abbe camera lucida. Figures 1 to 25, and figures 28, 34, 32, 36, and 37 are magnified approximately × 1200; all other figures are magnified approximately × 900.

PLATE 28

Fig. 1. Male and female branches.

Fig. 2. Later stage; the gametes are cut off from the basal cells. The cytoplasm of the oogonium is more dense than that of either the basal cell or the antheridial branch.

Fig. 3. The antheridial branch appears as if it had through more rapid growth pushed the oogonium to one side.

Fig. 4. The antheridial branch has completely overtopped the oogone.

Fig. 5. The antheridial cell is cut off from its stalk.

Fig. 6. The apex of the oogone shows a small densely stained area at the point of fusion with the oogone.

Fig. 7. An enveloping hypha is growing from the oogonial basal cell before fertilization has occurred.

Fig. 8. Antheridial nucleus has entered the oogonium; the pore has not yet closed.

Fig. 9. Stage just before conjugation. The male nucleus has enlarged and is about the same diameter as the female nucleus.

Fig. 10. Fusion has taken place; the conjugation pore has not yet closed and the densely stained area at apex of the oogonium persists.

Fig. 11. Shows the persistent densely stained area and large fusion nucleus.

Fig. 12. The enveloping hyphae in an early stage.

Fig. 13. Sex cells in an advanced stage in which the male nucleus has failed to enter the oogonium.

Fig. 14. Egg nucleus has divided; the densely stained area at the apex of the ascogonium is still present.

Fig. 15. Binucleated ascogonium with several enveloping hyphae growing from both male and female basal cells.

Fig. 16. Binucleated ascogonium with persistent densely stained area at the apex. Initial enveloping hyphae have completely covered the ascogonium and the second layer of hyphae is growing between it and the initial envelope.

Fig. 17. The hyphae of the initial envelope is cut up into several cells. The ascogonium has elongated slightly.

Fig. 18. The second layer is almost complete. Empty antheridial cell which has been pried off from the ascogonium by the enveloping hyphae is at the apex of the ascocarp.

Fig. 19. Empty male cell with its stalk embedded in the initial layer of the perithecium.

Fig. 20. An ascogonium with one very small nucleus and two equal sized larger nuclei, the latter are typical.

Fig. 21. Curved binucleated ascogone. The curve is perpendicular to the focal plane.

Fig. 22. Slightly older. The third layer of perithecial cells is nearly complete.

Fig. 23. Similar to fig. 21, but the ascogone is somewhat stouter.

Fig. 24. Both ascogonial nuclei have divided. The centripetal radial growth of the hyphae of the inner layer is shown.

PLATE 29

Fig. 25. Three-celled ascogonium. Stalk cell and basal cell are in the next section. The penultimate cell shows the two nuclei about to fuse.

Fig. 26. Fusion nucleus in the young ascus. The nucleoles have not yet fused.

Fig. 27. Young ascus with several small epiplasmic granules. The fusion nucleus shows several small blue granules.

Fig. 28. Young perithecium showing large granules in the older cells, smaller granules in the second layer and very few or none in the youngest the innermost cells. No epiplasmic granules are visible in the young ascogenous cell.

Fig. 29. The inner, nurse layer, is complete. The cells are large, inclined to be quadrilateral in section.

Fig. 30. Eight-spored ascus with numerous very small epiplasmic granules.

Fig. 31. Perithecium reconstructed from two sections. The basal cell is connected with the ascus stalk cell which contains four nuclei. The nurse layer has become considerably flattened by the growing ascus.

Fig. 32. Presumably a two-celled ascogone in which fertilization has failed to take place; enveloping hyphae have failed to develop.

Fig. 33. Later stage than fig. 31. Large blue epiplasmic granules in the ascus, and small dense granules in the outer cortical layer.

Fig. 34. Similar to 33, but stained with Unna blue. The epiplasmic granules remain unstained while the perithecial cell granules appear deep violet in color.

Fig. 35. Eight-spored ascus with thick, apparently gelatinized, wall. Large dark blue masses containing denser spherical bodies.

Figs. 36 and 37. Stages similar to fig. 32. Fig. 36a shows what might be an undeveloped antheridial branch.

Fig. 38. A four-celled ascogone with fusion nucleus in the young ascus.

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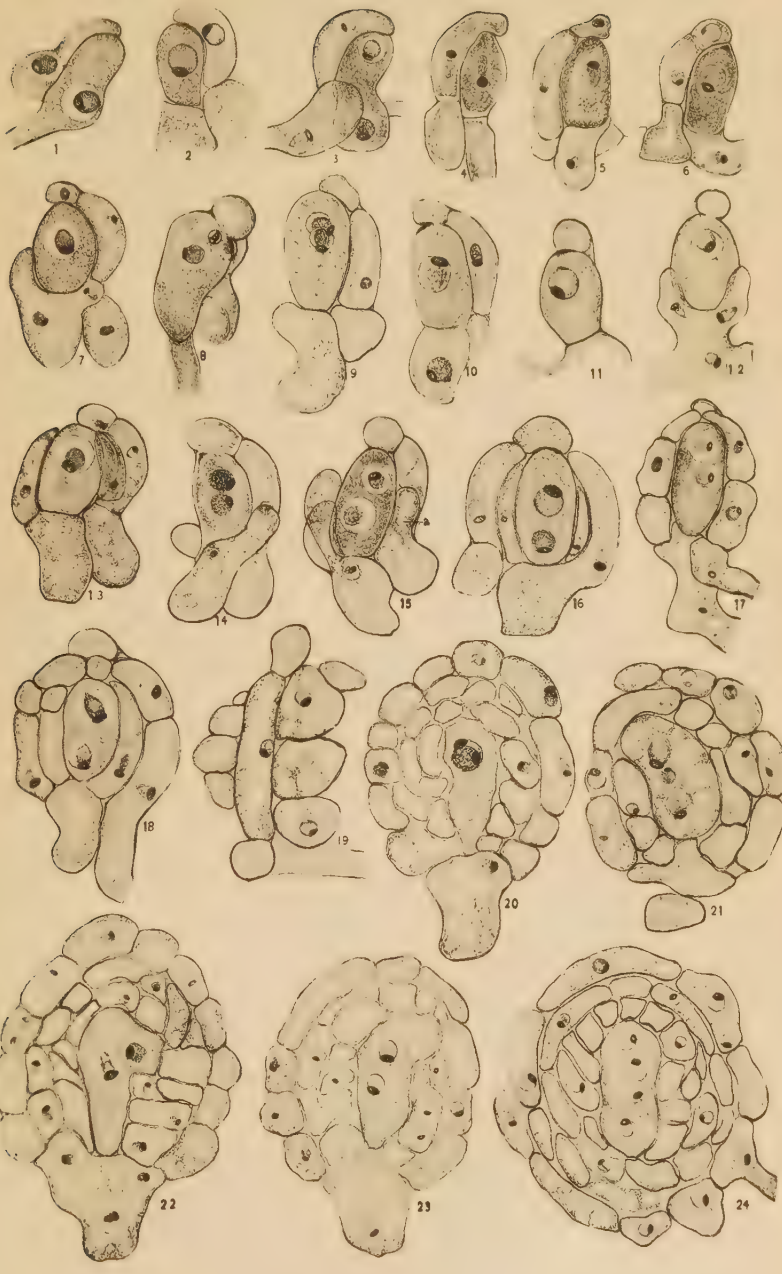
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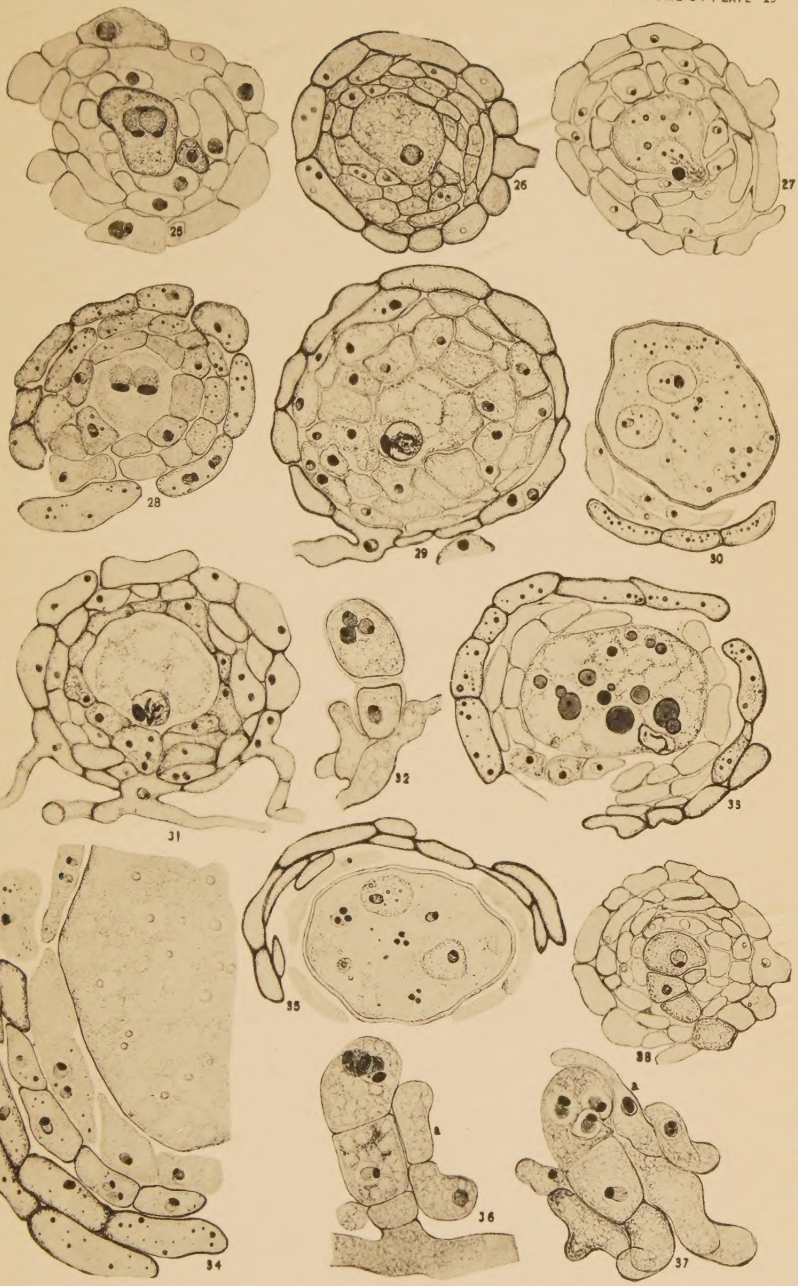
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